Chapter 1

Protein Translocation Across the Endoplasmic Reticulum Membrane

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Abstract

Proteins to be secreted from eukaryotic cells are delivered to the extracellular space after trafficking through a secretory pathway composed of several complex intracellular compartments. Secretory proteins are first translocated from the cytosol into the endoplasmic reticulum (ER), after which they travel by vesicular trafficking via various intermediate destinations en route to the plasma membrane where they are released from the cell by exocytosis. By sharp contrast, secretion in prokaryotes involves the translocation of proteins directly across the plasma membrane. While these two systems are superficially dissimilar, they are evolutionarily and mechanistically related. This relationship between the prokaryotic and eukaryotic systems of secretion forms the backdrop for this chapter focused on protein translocation into the ER. In the first part of this chapter, the essential steps and core machinery of ER translocation are discussed relative to evolutionarily conserved principles of protein secretion. The last section then explores the concept of regulation, a poorly understood facet of translocation that is argued to be evolutionarily divergent, relatively specific to the ER, and likely to be most highly developed in metazoans.

Reductionistic View of ER Translocation

The eukaryotic secretory pathway is thought to have evolved by a series of steps that were initiated by specialization of the prokaryotic plasma membrane (Fig. 1). This specialized region of membrane was then expanded, internalized, and eventually subdivided into many compartments. Hence, the lumenal space of compartments in the secretory pathway is topologically equivalent to the extracellular space, and the transport of proteins across the prokaryotic plasma membrane is directly analogous to transport into the ER. Both processes face the same basic challenges: (a) substrates to be transported need to be *recognized*, (b) selectively *targeted* to the site of transport, (c) vectorally *translocated* across the membrane, and (d) maintain a *permeability barrier* during these events. At the most fundamental level, these obstacles must have been solved in even the earliest life forms. This realization, together with the evolutionary relationship between the eukaryotic ER and bacterial plasma membrane, suggests a substantial conservation of the core principles of secretory protein translocation. Thus, assorted data using various model substrates from multiple systems (e.g., bacteria, archaea, yeast, and mammal) and multiple approaches (biochemical, genetic, and structural) have often been consolidated into

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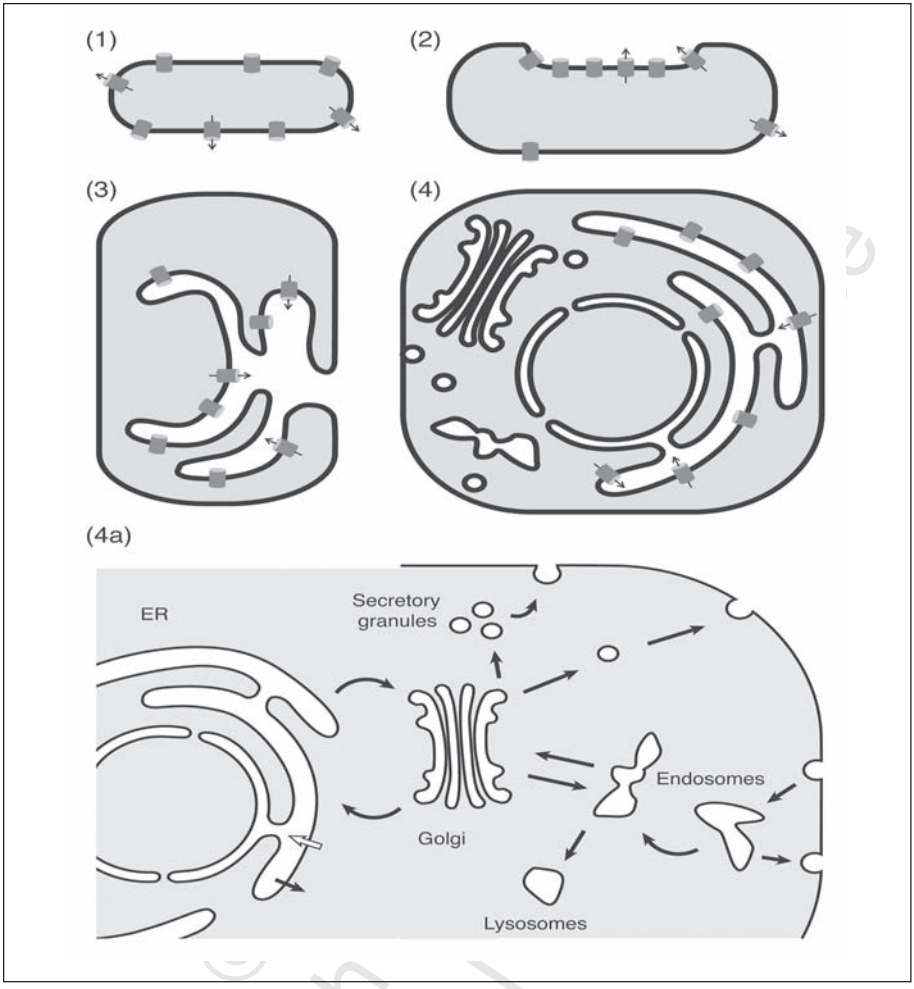

Figure 1. Evolution of the eukaryotic secretory pathway.
Steps (1) through (4) depict successive stages in the generally accepted view of eukaryotic secretory pathway evolution from a prokaryotic ancestor. The cytoplasm is shown in gray, and translocons for protein secretion are depicted by cylinders with the direction of polypeptide transport indicated by an arrow. Note the relationship between secretion across the bacterial plasma membrane (in stage 1) and translocation into the ER (in stage 4). Diagram 4a shows a more detailed view of the mammalian secretory and endocytic pathways, with the primary pathways of protein traffic indicated by arrows. Essentially all of these pathways have been discovered to be regulated in a manner that allows some, but not other substrates to be trafficked in appropriate amounts to meet the changing demands of the cell. Notable examples include quality control at the ER, exit from the ER, sorting at the Golgi, regulated exocytosis, and endocytic sorting and degradation. By contrast, translocation into the ER (open arrow) is often regarded as a constitutive process where the presence of a signal sequence in a protein predetermines its entry into the ER.

unifying models of protein translocation that are extrapolated to all systems.[1-4] While this provides a convenient framework for understanding protein translocation in general, it is apparent that further experiments will be required to either validate or revise the models for each individual system.

Basic Principles

Secretory and membrane proteins destined for the secretory pathway are recognized by the presence of hydrophobic domains in either signal sequences or transmembrane segments. N-terminal signal sequences (typically ~15-35 amino acids long) contain a hydrophobic core of at least 6 residues, while transmembrane segments have a hydrophobic stretch of between 16-25 residues. Aside from hydrophobicity, sequences used for the segregation of secretory and membrane proteins have no other features in common.[5,6] Indeed, the requirements are so degenerate that signals and transmembrane domains from prokaryotic and eukaryotic proteins are often functionally interchangeable,[7-9] and a surprising 20% of random sequences can at least partially mediate secretion from yeast.[10] Despite this tremendous diversity, signal sequences direct substrates into one of only two main translocation pathways in eukaryotes. In the cotranslational pathway (studied most extensively in the mammalian system), substrates are translocated across the membrane concurrent with their synthesis by membrane-bound ribosomes. In the post-translational pathway (studied primarily in the yeast system), the substrate is fully synthesized in the cytosol first, and translocated in a ribosome-independent fashion.

In cotranslational translocation, emergence from the ribosome of the first hydrophobic domain (either the signal sequence or transmembrane segment) allows its recognition in the cytosol by the signal recognition particle (SRP).[2,3] The complex of SRP and the ribosome-nascent chain (RNC) is then targeted to the membrane by an interaction with the SRP receptor (SR). At the membrane, the signal sequence is released by SRP, the RNC is transferred to the translocon, and the SRP-SR complex is dissociated. Thus, the targeting cycle culminates with delivery of the RNC to the translocon and recycling of components of the targeting machinery (SRP and SR) for the next substrate.

Nascent chains that are cotranslationally targeted to the translocon must then engage the translocation channel, mediate its opening, and be transported through it across the membrane. The central component of the translocation channel is the evolutionarily conserved heterotrimeric Sec61 complex.[11,12] The Sec61 complex, which has a high affinity for ribosomes,[13] provides a docking site for RNCs without the need for other components. However, docking of an RNC at the translocon is not sufficient to initiate translocation. Rather, engagement of the channel requires a functional signal sequence (or transmembrane domain), whose association with the Sec61 complex represents a second substrate recognition event during cotranslational translocation.[14]

This second recognition step may serve a 'proofreading' purpose to ensure that no nonsignal containing substrates that inadvertently target to the channel can engage it. More importantly, binding of the signal to the Sec61 complex triggers at least three essentially simultaneous changes in the RNC-translocon complex: (a) an increase in stability of the interaction between the RNC and translocon, (b) insertion of the nascent chain into the translocation channel, and (c) opening of the translocation channel towards the lumen.[14-18] Upon successful completion of these steps, the substrate resides in a continuous path running from the peptidyl transferase center in the ribosome, through the translocation channel, and into the ER lumen.[14,15,19] From this point, continued protein synthesis is thought to result in 'pushing' of the nascent chain through the channel and across the membrane. Hence, the architecture of the RNC-translocon complex[20-22] biases the direction of nascent chain movement, thereby harnessing the energy of protein synthesis to simultaneously drive translocation.

Post-translational translocation operates in several qualitatively different ways. In eukaryotes, this pathway has been studied most extensively in yeast, where a seven protein Sec complex at the ER membrane and the lumenal chaperone BiP (known as Kar2p in yeast) have been identified as the essential translocation apparatus.[23-27] This Sec complex can be conceptually (and experimentally) divided into two sub-complexes: the trimeric Sec61 complex (homologous to the mammalian Sec61 complex), and the tetrameric Sec62/63 complex.[23] The Sec61 complex presumably forms a similar channel in the post-translational Sec complex as it does in the cotranslational translocon.[28] This means that the remaining components (the Sec62/63 subcomplex and BiP) must fulfill the functions otherwise provided in cotranslational translocation by the targeting machinery (SRP and SR) and ribosome, neither of which are involved in post-translational translocation.

Consistent with this idea, the Sec62/63 complex (but not BiP) is essential for signal sequence recognition by the Sec61 complex.[23,27,29] Thus, the Sec complex, by selectively binding signal-containing substrates, mediates targeting to the translocon in a single mechanistic step that replaces the series of targeting reactions involving the ribosome, SRP, SR, and translocon. Once substrate is bound to the Sec complex, the Sec61 translocation channel is thought to be engaged and opened in a similar fashion to the signal-mediated gating step in cotranslational translocation.[30] The substrate would then need to be moved unidirectionally through the Sec61 channel across the membrane.

Since vectorial movement of the substrate through the channel cannot exploit the energy of protein synthesis (as during cotranslational translocation), the actual transport step needs to occur differently. This function of biasing the direction of polypeptide movement is provided by BiP, a chaperone that binds the substrate on the lumenal side of the translocation channel to prevent its back-sliding into the cytosol.[23,25-27,31] Subsequent rounds of binding and release, stimulated by ATP hydrolysis, allows BiP to act as a molecular ratchet to drive substrate transport into the lumen.[32] The ATPase activity of BiP is regulated by Sec63p, a J-domain containing component of the Sec complex, which presumably also serves the function of recruiting BiP to the translocation channel.[27,33] Thus, the substrate is largely 'pulled' across the membrane from the lumenal side in the post-translational pathway, in contrast to being 'pushed' from the cytosolic side in cotranslational translocation.

A comparative analysis of these basic features of eukaryotic cotranslational and post-translational translocation reveals an important central theme (Fig. 2). It has become clear that the actual channel through which the polypeptide is translocated acts as a relatively passive conduit. It only acquires its functionality for substrate recognition and vectorial transport upon interaction with various binding partners. In cotranslational translocation, a key binding partner is the ribosome which acts to mediate translocon assembly, 'primes' the Sec61 complex for signal recognition, and couples the energy of protein synthesis to translocation. In post-translational translocation, the key binding partner is the Sec62/63 complex which, like the ribosome, facilitates translocon assembly, allows signal sequence recognition, and provides the driving force for translocation by recruiting and regulating the function of BiP at the translocation site. Indeed, even in the bacterial system, the homolog of the Sec61 complex (termed the SecY complex) interacts with the cytosolic SecA ATPase that both receives the substrate at the channel and drives its subsequent translocation across the membrane.[34] Thus, the highly conserved Sec61 channel can be exploited in several markedly different ways by various coassociating partners that mediate protein translocation across the eukaryotic ER or prokaryotic plasma membrane.[1,4]

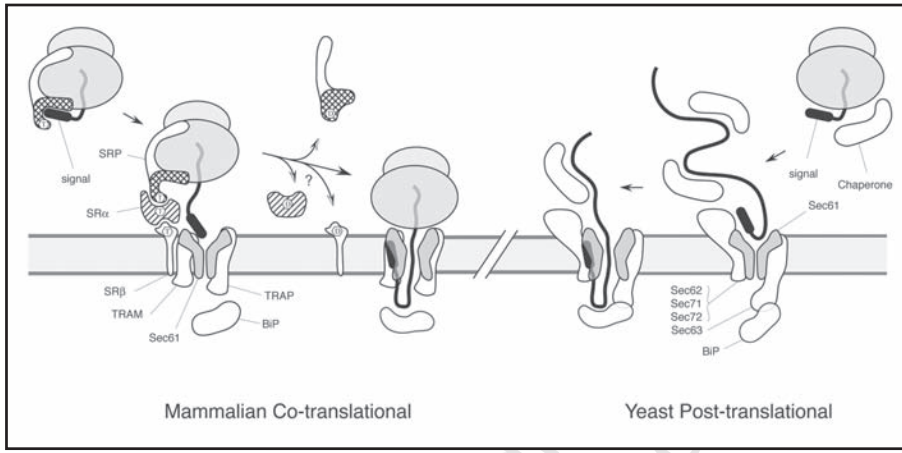

Figure 2. Pathways of ER protein translocation.
The principal machinery and steps of the eukaryotic cotranslational and post-translational pathways are shown on the left and right, respectively. The components of each pathway that are conserved in all organisms (in both prokaryotes and eukaryotes) are shaded, and include the signal sequence, ribosome, SRP54 (along with a portion of its associated SRP RNA), SRα, and the Sec61 complex. Various other components that function in each pathway are also shown. The GTP- and GDP-bound states of the cotranslational targeting machinery are displayed with 'T' and 'D' respectively. The center two diagrams depict the comparable 'committed' stages of the two pathways to illustrate that in both, the Sec61 complex serves the same passive role as the channel while the associated components function to keep the polypeptide unfolded and move it vectorally into the lumen.

Molecular Details

Signal sequence recognition and targeting is understood in the greatest molecular detail for the cotranslational (i.e., SRP-dependent) pathway in eukaryotes. This is largely because the remarkable evolutionary conservation of this pathway from bacteria to mammals has allowed the experimental results from multiple systems and approaches to be combined.[3] In higher eukaryotes, SRP is a ribonucleoprotein composed of six proteins (named by their apparent molecular weights: SRP72, SRP68, SRP54, SRP19, SRP14, and SRP9) and a ~300 nucleotide RNA (termed 7SL RNA or SRP RNA).[35,36] Of these components, SRP54 and a portion of the RNA are directly involved in both signal sequence recognition and the interaction with SR. Indeed, these two components define the minimal SRP that can be found in all organisms of every kingdom of life.[3] In most, if not all bacteria, *only* these two components are found, indicating that they can perform all of the recognition and targeting functions necessary for translocation.[37-41]

Structural analysis of SRP54 homologues from several organisms[42-48] has revealed that it is universally organized into three functional segments: the M-, N-, and G-domains. Of these, the M-domain recognizes signal sequences via a deep, hydrophobic groove lined by the flexible side chains of several methionines. Phosphates of the RNA backbone are near one end of this groove, and may interact with basic residues that are often (but not always) adjacent to the hydrophobic core of signal sequences and transmembrane domains. These and other conserved features of SRP54 help to explain how it can accommodate a wide range of signal sequences whose only common feature is a hydrophobic segment, and why signals from diversely different organisms are often interchangeable.

In addition to signal sequence recognition, the other essential function of SRP is its interaction with SR to ensure the targeting of nascent secretory and membrane proteins to the translocon. The tight coordination of the series of interactions that imparts unidirectionality to the targeting phase of translocation is through the regulated GTPase activities of SRP and SR. The GTPase component of SRP resides in the G-domain of SRP54 (ref. 35). In eukaryotes, SR is a heterodimer of α and β subunits,[49] both of which are GTPases.[37,50] Of these, SRα is highly conserved from prokaryotes to mammals and, together with SRP54 and SRP RNA, represents the minimal targeting machinery found in all organisms.[37,41] Detailed mechanistic and structural analysis of this minimal SRP pathway, mostly using the model bacterial system, has revealed the essential aspects of their regulation during cotranslational targeting.

In the current working model, free SRP in the cytosol is in the GDP-bound state. Its association with the ribosome stimulates GTP binding,[51] and subsequent association with the signal sequence inhibits GTP hydrolysis.[52,53] Thus, the signal-SRP-ribosome ternary complex is likely to be in the GTP-bound state. Although less direct evidence exists for SRα, it is thought that its association with a vacant translocon at the membrane (directly in the case of prokaryotes, and indirectly via SRβ in eukaryotes) may similarly allow GTP binding and prevent GTP hydrolysis. Thus, the SR-translocon complex would also be in the GTP-bound state. The GTP-bound forms of SRP54 and SRα have a high affinity for each other,[53] allowing the delivery of signal-containing RNCs to the close proximity of an appropriately vacant translocon.[54,55]

The interaction between the GTPase domains of SRα and SRP54 stimulate the hydrolysis of GTP by each other (thereby acting as GTPase activating proteins, or GAPs, for one another).[56] The change in conformation that accompanies this GTP hydrolysis results in a weakening of the interaction between SRα and SRP54, allowing this complex to be dissociated for another round of targeting.[54,57,58] Many of the molecular details of this generally appealing scheme remain to be elucidated. For example, SRP RNA,[59,60] as well as the translocon[61,62] and the ribosome,[51] clearly facilitate aspects of SRP-SR interactions and their GTPase activities. However, the precise mechanisms remain elusive at the present time. The recently emerging wealth of structural information on SRP and SR should help to illuminate the molecular details of this framework.

Beyond these essential functions performed by the minimal components, the significantly more complex eukaryotic SRP and SR are likely to confer additional functionality and advantages for the cell. One such eukaryotic-specific feature is the slowing of translation upon signal sequence binding by SRP, a phenomenon termed 'elongation-arrest'.[63,64] The mechanism appears to involve occlusion of the elongation factor binding site on the ribosome by the SRP9 and SRP14 subunits of SRP.[65] The resulting decrease in translational rate serves to increase the time available for targeting to the translocation channel before excessive polypeptide synthesis precludes cotranslational transport. While translational attenuation by SRP is not essential for translocation,[66] it appears to be physiologically important under at least some growth conditions in vivo.[67] Whether the other subunits of SRP (SRP68, SRP72, and SRP19), each of which is important for assembly (particularly SRP19) and stability of the complete particle,[68] confer yet additional functionality to eukaryotic SRP remains largely unknown. Similarly, SRβ, a homolog for which does not exist in prokaryotes, is likely to provide the bridge that further regulates the coordinated transfer of RNCs from SRP to the translocon. This appears to be accomplished by the regulation of SRβ GTPase activity by both the ribosome[69] and the translocon,[62] with accompanying conformational changes that are suggested to affect the RNC-SRP54-SRα-SRβ interactions.[70]

Signal sequences and transmembrane domains are also recognized by the translocon at the membrane in all modes of translocation.[14,30,71-73] The purpose of this recognition is two-fold. First, it provides a mechanism for discriminating translocation substrates from other proteins.

This is the sole discriminatory step in post-translational translocation, and a secondary (or 'proofreading') step in cotranslational translocation. Second, signal recognition by the translocon is essential for its opening (or gating) in preparation for substrate transport.[14,73] Since the core of the translocation channel in both co and post-translational translocons of both prokaryotic and eukaryotic cells contains the Sec61 complex, the basic mechanism of signal recognition at the membrane in all cases is presumed (but not yet demonstrated directly) to be mechanistically similar. Hence, this step would appear to be a point of convergence for both co- and post-translational translocation pathways in different organisms. Indeed, recognition of signals (and transmembrane domains) by the Sec61 translocation channel is likely to be as ancient and evolutionarily conserved as signal recognition by SRP. Yet, the Sec61-mediated recognition step, by striking contrast to SRP-mediated recognition, is very poorly understood. This is in part because the membrane proteins involved in translocon signal recognition are significantly more difficult to manipulate and study, relative to the cytosolic SRP. However, if one is allowed some degree of extrapolation across species, a general framework and a few mechanistic details of signal recognition by the translocon can be compiled.

Cross-linking studies in both mammalian and yeast systems suggest that the signal sequence binds to a site that is at the interface of the Sec61 channel and the surrounding lipid bilayer.[30,71,74] Detailed analysis of the regions of yeast Sec61p that interact with the signal sequence of a model substrate (prepro-α factor) has implicated transmembrane helices 2 and 7 as forming the binding site.[30] These same two helicies of the bacterial SecY complex were also observed to interact with a synthetic signal peptide in detergent solution.[72] All of these findings from the mammalian, yeast, and bacterial systems can now be reconciled with the crystal structure of an archaeal SecY complex.[75,76] This structure revealed that helicies 2 and 7 are indeed adjacent to each other and provide a lateral exit site from the proposed pore within SecY to the lipid bilayer.[76] Thus, it seems reasonable to conclude that in all systems, signal sequences (and transmembrane domains) of translocation substrates are recognized by a site in Sec61/SecY that is composed of the two transmembrane helicies (2 and 7) that line the lateral exit site from the translocation channel.

In addition to this 'generic' signal recognition site in Sec61, it is clear that other Sec61-associated components are also involved in signal recognition in many cases. These additional components may, directly or indirectly, stabilize signal sequence-Sec61 interactions for at least a subset of substrates. In cotranslational translocation, these additional components include the TRAM protein[77,78] and the tetrameric TRAP complex.[79] In both cases, these accessory translocon components are required in a signal sequence-dependent manner for the translocation of some, but not other substrates. TRAM has been directly implicated in interacting with the hydrophilic region that directly precedes the hydrophobic core of a signal peptide.[80] The role of TRAP is less clear, but it may act indirectly by stabilizing the Sec61 channel with which it directly interacts. In post-translational translocation, the Sec62/63 complex is absolutely required for signal sequence recognition by the Sec61 complex.[23] The mechanism is not yet clear, but it may be a combination of direct signal sequence interactions (e.g., with Sec62, which has been implicated in cross-linking studies[27,30,81]), or indirect effects as a consequence of stabilizing the Sec61 translocon.[28]

The features of the signal that determine the need for these additional components are not well-studied, nor are the mechanisms by which they facilitate recognition. Furthermore, whether yet other components are also involved in substrate-specific aspects of signal recognition is also not known. Numerous proteins, particularly in the mammalian system, have been identified to be at or near the site of translocation. These include proteins with known functions (such as the multi-protein oligosaccaryl transferase complex[82] or five protein signal peptidase complex[83]), as well as many others whose functions are not known.[84-87] While none of these are absolutely essential for translocation of at least the simplest model substrates,[12] it is not known

whether they play essential or stimulatory roles in translocation of select substrates. As was exemplified by the TRAP complex, the functions of such accessory factors may elude detection[88] unless the proper substrate is examined.[79]

Maintaining the Membrane Permeability Barrier

During protein translocation, the membrane permeability barrier to the passage of small molecules should not be compromised. How this is achieved remains a matter of considerable debate. It is clear, however, that resolving this issue will require information about the architecture of the translocon, the structure of its individual constituents, and how they are assembled and changed during the functional translocation cycle. This will provide critical information about the nature of the translocation pore, its size, how it might be opened and closed, and how its permeability to small molecules can be controlled both during and in the absence of substrate translocation. At present, such structural and organizational information about the translocon and the pore are only beginning to emerge, leaving the mechanism of membrane permeability maintenance unresolved.

The first experimental studies to begin addressing the issues of pore size and membrane permeability were in the mammalian cotranslational system. In these experiments, translocation intermediates were assembled in which the substrate contained within it a fluorescently labeled amino acid at a defined position. The fluorophore was then used as a probe of both the environment surrounding the nascent chain[89] and the accessibility of this environment to exogenously added molecules capable of quenching the fluorophore.[15,90-92] The ability to control substrate length (and hence, the stage of translocation), the position of the probe, and the size and location of the fluorescence quenchers allowed various parameters of the translocon to be deduced. From these studies,[93] the pore sizes of inactive versus engaged translocons were measured to be ~8-10 Å and ~40-60 Å, respectively.[90] Preventing the passage of small molecules through this pore depended on alternately sealing the channel with either a ribosome on the cytosolic side or BiP on the lumenal side.[90-92] Sequences in the nascent polypeptide are proposed to choreograph the dynamics of channel gating by the ribosome and BiP to allow substrate transport without small molecule leakage.[92] Recently, an electrophysiological approach also suggested that purified Sec61 complex in lipid bilayers may contain pores as large as 60 Å that can be blocked by BiP.[94]

Although the model derived from the fluorescent probe approach is internally consistent and compatible with many other biochemical experiments in the mammalian cotranslational system, several arguments against it have been raised. In one experiment, the inability to detect folding of even a small domain while it is inside the translocon[95] seemed at odds with the proposed 40-60 Å pore size.[90,94] However, it is not clear how generalizable the results from either approach are since in each case, a single (and different) substrate has been examined to measure pore size. In other experiments, structural studies using cryo-electron microscopy (EM) of RNCs bound to the translocon failed to see a tight seal between the ribosome and translocation channel that was expected from the fluorescence quenching studies.[20-22,96] However, an inability to see density by cryo-EM can be difficult to interpret since it could be due to increased flexibility in those regions of the structure, loss of ancillary translocon components upon solubilization and sample preparation, or sample heterogeneity. Thus, cytosolic or membrane components in addition to the ones visualized by cryo-EM may form the putative seal between the ribosome and membrane. Indeed, several abundant membrane components have been identified associated with the translocon (some with large cytosolic domains such as p180)[84] whose functions remain unclear. Thus, there are some potentially plausible ways to reconcile much of the seemingly conflicting data gathered on membrane permeability and translocon architecture of the mammalian cotranslational system.

More problematic however is the argument that the proposed mechanism involving the ribosome and BiP during mammalian cotranslational translocation does not shed light on how the permeability problem is solved in other modes of translocation or in bacterial systems. In the post-translational pathway, the ribosome is not involved in translocation, precluding a role for it in maintaining the permeability barrier. In Bacteria, it is unclear what would serve the function of the lumenal gate proposed for BiP in the mammalian system. Because of these difficulties, a more generally applicable and evolutionarily conserved solution to the permeability barrier problem has been sought. The most insight into such a putatively conserved mechanism comes from interpretation of the recent high resolution crystal structure of an archaeal SecY complex.[75,76]

In this structure, a single SecY complex was found to form a channel-like structure with a very small pore flanked on the lumenal and cytosolic sides by funnels. The narrow constriction between these two funnels is only ~5-8 Å in diameter and lined by several hydrophobic residues that together form the 'pore ring.' If the channel formed by a single SecY complex is the functional pore through which the substrate is transported, the small size and flexibility of the 'pore ring' side chains would then form a relatively snug fit around a translocating polypeptide. This mechanism of translocation would solve the permeability problem because the nascent chain itself can occlude the channel during translocation. Furthermore, another small segment of the SecY protein (termed the 'plug' domain) appears to occlude the pore in its inactive state.[76] Thus, no additional components would be required to maintain permeability except the Sec61/SecY complex, which forms the channel in all modes of translocation.

The theoretical and experimental evidence that the translocation pore is indeed formed within a single SecY or Sec61 complex, despite its oligomerization into a larger structure,[22,28,97,98] is reviewed in detail elsewhere.[75,76] In essence, it is argued that a hydrophilic pore cannot be formed at the interface of multiple SecY complexes unless they 'face' each other, a configuration the authors of the structural work consider unlikely based on experiments examining the bacterial SecY complex.[99-102] Whether this proves to be true in all translocation systems, and hence explains the permeability problem, remains to be investigated. The alternative explanation is that in eukaryotic systems, the basic unit of translocation has evolved into a more malleable oligomeric structure in which the pores of multiple Sec61 complexes can indeed be combined to form a larger translocon that changes to meet the demands of the substrate.

This explanation would necessitate additional protein complexes that facilitate this reorganization and new mechanisms to solve the permeability problem. While this might seem unnecessarily complicated, it is not unreasonable given the existence of numerous eukaryotic-specific translocation components whose functions remain largely unknown (such as Sec62, Sec63, TRAM, or TRAP, among many others) At present, the choice among the different views depends largely on where a philosophical line is drawn. On the one hand is the tremendous degree of evolutionary conservation of the most fundamental features of protein translocation that has allowed information across multiple kingdoms to be combined into explanations applicable to all systems. On the other hand is the equally powerful feature of evolution to forge new biological principles using the same basic constituents. Clearly, the former is justified when one considers examples such as the SRP pathway, while the latter is strikingly exemplified by the evolution in eukaryotes of mechanisms to 'pull' nascent chains across the membrane from a system initially designed to 'push' such chains from the cytosolic side. Ultimately, experimental results will be needed to resolve these issues and determine the degree to which evolution has been conservative versus inventive in shaping eukaryotic protein translocation across the ER.

Regulation of Translocation

The evolution of a complex endomembrane system in eukaryotes (Fig. 1) provides several advantages to the cell, some of which are more obvious than others. These advantages include increased capacity, quality control, quantity control, and regulation. In this last section, examples of the ways in which the development of a multi-compartment secretory pathway has been exploited in complex eukaryotic organisms is discussed as a means of illustrating a general principle of regulated biological processes. This principle is then used to develop a rational framework for how and why eukaryotic protein translocation is likely to be a highly regulated process. And finally, the (albeit limited) data on translocational regulation is compiled into some potential mechansims by which ER protein translocation can be controlled in a substrate-specific manner.

The first advantage of compartmentalization, increased capacity, is a direct consequence of the substantially increased surface area of membrane across which a protein can be translocated. In the most extreme instance, the ER is expanded to almost completely fill the cells of highly secretory tissues such as the exocrine pancreas. The increased surface area (and hence capacity) conferred by the secretory and endocytic pathways is one reason (among many) that eukaryotic cells can be substantially larger than prokaryotes. The other three advantages are inter-related, and all a direct consequence of the fact that secretion in eukaryotes is a multi-step process that begins, not ends with translocation across the membrane. Thus, upon translocation, the protein is still available to a eukaryotic cell before its secretion, while in prokaryotes, translocation is largely synonymous with exit from the cell. This availability has been thoroughly exploited to confer several important advantages to eukaryotes.

The most important advantage is the opportunity for quality and quantity control: since a translocated protein in eukaryotes is not lost to the extracellular space, there is time to impose a 'recall' in instances where the protein is not desired. Hence, if a protein is not matured or assembled properly, it is rerouted for degradation (i.e., quality control),[103,104] thereby avoiding the potentially detrimental consequences of misfolded or incomplete secretory and membrane proteins. This has almost certainly facilitated the evolution of very complex secretory proteins (such as apolipoprotein B) or multi-component membrane protein complexes (such as the T-cell receptor). Similarly, regulated degradation after translocation allows the abundance of secretory or membrane proteins to be modulated in response to need (i.e., quantity control, exemplified by HMG-CoA reductase[105] or apolipoprotein B[106]). Furthermore, the intracellular compartmentalization of secretion allows secretory and membrane proteins to be stored until they are needed,[107] at which point they can be rapidly delivered to selected regions of the cell surface by exocytosis. Thus, secretion of extracellular proteins or surface expression of membrane proteins can be rapid, quantal, and temporally and spatially regulated. These examples illustrate an important general principle: the disadvantages of increased cost and lower efficiency of a more complex, multi-step process (e.g., the secretory pathway) can be offset by the benefits of a greater degree of regulatory control. Thus, potentially regulatory aspects of the secretory pathway are likely to be most thoroughly developed in systems where control, and not just energetic cost, is of the utmost importance.

In which organisms is the highest premium placed on precise control of secretory and membrane protein biogenesis? The answer is multicellular organisms, whose fitness depends not only on the health of individual cells, but equally (or perhaps even more) on the ways those cells interact, communicate, and function as complex units. Such communication and interactions are intimately dependent on secreted and cell surface proteins whose amounts at the right time and place must be carefully regulated. Thus, completely healthy *individual* cells in a complex organism can nonetheless lead to failure of the organism if they do not function coordinately in extremely precise ways. Countless examples of this idea can be found in human

physiology and disease, including the regulation of blood pressure, reproductive cycles, stress, appetite, and weight regulation. It should, therefore, come as no surprise that *each and every step* in the secretory pathway that has been examined was discovered to be regulated to tightly control the levels of secretory and membrane proteins in response to cellular and organismal needs (Fig. 1). Will protein translocation prove to be any different, once more complex (and subtle) aspects of this process have received experimental attention? Almost certainly not.

How then might one conceptualize a framework for translocational regulation that can guide future investigation? At the outset, it is instructive to consider analogies to other regulatory systems for common themes that can be applied to translocation. In this vein, a grossly simplified discussion of transcriptional promoters and their regulation is useful[108-111] (although similar arguments can be made equally well with any other regulatory process). In transcription, sequence features that are common to all promoters are accompanied by sequence elements that are unique to each individual promoter.[112] Thus, each promoter is unique, but contains at least some common elements that allow it to be recognized as a promoter per se. The common elements allow a core (or 'general') machinery to mediate transcription,[108,109] while the unique elements impose requirements for additional machinery that regulate the recruitment or activity of the core components.[110,111] The combinatorial expression or modification of the unique machinery can dramatically influence the activity of any given promoter. By regulating individual components of the unique machinery in a temporal or cell type-specific manner, transcriptional regulation of individual promoters can be achieved independently of each other. Thus, sequence diversity of promoters combined with diversity in the components that recognize them allows selective regulation of genes that all, nonetheless, use a commonly shared core machinery for transcription.

Applying this general idea to translocation allows at least one mechanism of regulation to be conceptualized. Here, signal sequences are viewed as loosely analogous to promoters, and the evolutionarily conserved components of the translocation machinery (i.e., SRP, SR, and Sec61 complexes) are analogous to the core transcriptional machinery. Signal sequences are indeed extremely diverse, with each substrate containing an effectively unique signal, while nonetheless sharing certain common, recognizable features.[5,6] The common features of the signal appear to be the elements that are recognized by the core machinery, such as SRP54 and the Sec61 complex. The unique features of the signal appear to impose additional constraints on signal function by requiring the presence of additional factors at the translocation site such as TRAM or the TRAP complex.[77-79] These additional components can be modified (e.g., by phosphorylation[113-115]), which potentially may selectively modulate their activity (although this has yet to be examined). Thus, even using only the limited information that is currently known, one can easily envision the basic elements of a substrate-specific system of translocational regulation (Fig. 3): (a) diversity in structure and function of signal sequences that share a bare minimum of common features, (b) diversity in 'accessory' components that influence recognition by a core translocation machinery of some, but not other signals, and (c) selective changes in expression or modification of the 'accessory' components that could affect the outcome of translocation for some, but not other substrates.

This view of regulating translocation by the combinatorial functions of accessory components can be readily expanded to incorporate the many other factors at or near the site of translocation whose functions remain elusive. In the mammalian system, these include Sec62, Sec63, p180, p34, a TRAM homolog, and yet unidentified proteins observed by cross-linking studies. Each of these components could potentially play stimulatory (or inhibitory) roles in the translocation of selected substrates, with the specificity encoded in the sequence diversity of the signal. Such accessory components can not only be modified, but themselves regulated at steps such as alternative splicing[116] or differential expression[117] to influence their function.

A

Protein	Signal sequence	Length	Charge
BiP	MKLSLVAAMLLLLSAARA	18	+1
Prolactin	MNIKGSPWKGSLLLLLVSNLLLCQSVAP	28	+2
TGF-β2	MHYCVLSAFLILHLVTVAL	19	0
TGF-β3	MKMHLQRALVVLALLNFATVSLS	23	+2
Growth hormone	MATGSRTSLLLAFGLLCLPWLQEGSA	26	+1
Interferonγ	MKYTSYILAFQLCIVLG	17	+1
Osteopontin	MRIAVICFCLLGITCA	16	+1
Leptin	MHWGTLCGFLWLWPYLFYVQA	21	0
ANP	MSSFSTTTVSFLLLLAFQL	19	0
Apo-A1	MKAAVLTLAVLFLTGSQA	18	+1
EGF-receptor	MRPSGTAGAALLALLAALCPRA	24	+1
Glucagon	MKSIYFVAGLFVMLVQG	19	+1
Inhibinβ	MPLLWLRGFLLASCWIIVRSSPTPGS	27	0
HLA-DCβ	MSWKKALRIPGGLRVATVTLMLAMLSTPVAEG	32	+2
Choriogonadotropin	MEMFQGLLLLLLLSMGGTWA	20	-1

B

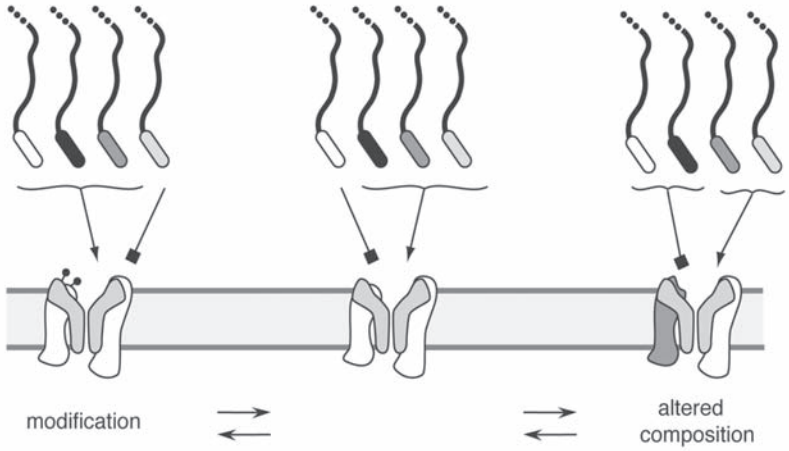

Figure 3. Conceptual framework for translocational regulation.
One potential mechanism for regulating the translocation of secretory and membrane proteins into the eukaryotic ER is shown. Here, the sequence diversity of signals can be exploited to selectively influence the translocation efficiencies, and hence functional expression, of some but not other secretory pathway proteins. The key discriminatory step is proposed to occur at the translocon, whose modification or composition would affect its selectivity for the range of signal sequences that it can recognize to initiate translocation. Thus, the encoding of a signal sequence in a protein is not viewed as a guarantee of its entry into the secretory pathway; rather, the signal is a provisional 'license' for translocation that is contingent on the translocon, whose functional state can be modified in response to cellular need. Panel A shows a representative sample of several signal sequences from human secretory proteins. Note the wide diversity of amino acid sequence, composition, length, and charge of the N-terminal domain preceding the hydrophobic sequence. Panel B illustrates the concept of translocational regulation described above. In this example, several substrates that differ in their signal sequences are either accepted (arrow) or rejected (square) by each of the hypothetical translocons. Note that the selectivity is altered upon changes in either the modification state (left) or composition (right) of the translocon machinery.

Thus, there exist more than enough sources for modulatory activities to theoretically provide exquisite specificity in the regulation of signal sequence function, and hence translocation.

Initial evidence that protein translocation can indeed be modulated in a substrate-selective, cell-type specific way has recently been provided by quantitatively examining the efficiency of signal sequence function in vivo.[118] Not only were different signal sequences found to have different efficiencies within a given cell type, but they also varied independently in a cell type-specific manner. For example, one signal sequence was observed to be significantly more efficient than another signal in a particular type of cell; in a different cell type, the two signals were found to be equally inefficient. Thus, the entry of proteins into the ER is not necessarily a constitutive process predestined by the sequence of the substrate. Rather, it is dependent on and potentially regulated by the machinery that mediates its translocation.

Consistent with this view, are the numerous examples in which secretory pathway proteins are also found in alternative locations (summarized in ref. 118), the extent of which can vary in a cell type-specific manner. The mechanistic basis or physiologic relevance of these observations is not yet clear, but at least some of these examples are likely to be a consequence of translocational regulation. Clearly, modulating translocation not only provides one means of quantity control (i.e., the ability to change the abundance of the protein in the secretory pathway), but also a mechanism to generate an alternative form of the same protein in another compartment, where it could potentially serve a second function. Examples of proteins that may have such alternative functions in different compartments have been suggested (see ref. 118 for a summary). The degree to which translocational regulation is beneficially utilized for the generation of functional diversity or quantity control of secretory pathway proteins remains to be investigated.

Conclusions

The eukaryotic translocation pathways contain a very well-recognizable core machinery whose identity and mechanisms of action are remarkably well-conserved across all kingdoms of life. The signal sequences and transmembrane domains that engage this machinery are essentially indistinguishable when comparing the prokaryotic versus eukaryotic populations of substrates. Despite these similarities in substrate clientele, the core translocation machinery has been embellished at every conceivable step during the evolution of eukaryotes (Fig. 2). The reasons for this increase in complexity, which are probably varied and numerous, remain poorly understood. One general explanation may be that a higher premium is placed in more complex organisms on fidelity of protein biogenesis. Not only are proteins generally more complex and multi-domained in eukaryotes, but the consequences of their misfolding may be more detrimental in cells whose proper function depends on a larger set of intersecting biochemical pathways. This may be particularly important in highly differentiated cells of multi-cellular organisms that must live long periods of time without replacement by cell division. Thus, very tight control of targeting and translocation, with contingencies for errors at each step, may provide subtle advantages to eukaryotes that outweigh the costs of increased complexity and energy expenditure. A more important reason for the multiple layers of complexity during eukaryotic translocation may be to facilitate cellular control at each step. This general theme of embellishing a basic process to allow for regulation is seen in virtually every other cellular process such as transcription, translation, or cell division. By adding accessory components whose activities can be used to modulate a core machinery, a biological process such as transcription can be changed in response to cellular demand or environmental conditions. If and how protein translocation can be regulated remains essentially unexplored at the present time, but is envisioned to utilize themes common to other biological regulatory processes. This concept of translocational regulation represents a fertile area for future research in this field.

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